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Microglial endocytosis and transglutaminases

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Abstract

Microglia categorized into macrophage is considered to be a major cell for immunity in central nervous system. Activated microglia release nitric oxide (NO) and proinflammatory cytokines to damage neurons, and also produce neurotrophins to protect neurons. In addition, activated microglia enhance endocytotic activity to engulf invading microorganisms and scavenge cell debris and damaged cells. Engulfment of neurons by activated microglia may play roles in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Tissue-type transglutaminase (transglutaminase 2; TG2) is a cross-linking enzyme, which is activated in AD, PD and Huntington's diseases. TG2 is involved in connection to phagocytes with apoptotic cells. In the present review, we explained in microglia the endocytosis mechanisms and the involvement of TG2 in endocytosis to be associated with neurodegenerative diseases.

Introduction

In pathological mechanism of various neurodegenerative diseases, it is well known that the activation of glial cells play important roles for both exacerbation and recovery [1-3]. Therefore, the elucidation of the mechanism of glial activation is essential to overcome such diseases. Astrocytes play various important roles in central nervous system (CNS), such as maintenance of blood brain barrier, scavenging some neurotransmitters, control of ionic balance in brain parenchyma. These functions of astrocytes serve the maintenance of brain homeostasis [4-6]. Functional changes of astrocytes are involved in neurodegenerative processes in various CNS diseases including Alzheimer's diseases (AD).

Microglia categorized into macrophage is considered to be a major cell for immunity in CNS. Activated microglia release nitric oxide (NO) and proinflammatory cytokines to damage neurons, and also produce neurotrophins to protect neurons. In addition, they engulf invading microorganisms and scavenge cell debris and damaged cells [1,2,7,8]. Microglial phagocytosis contributes to maintaining of homeostasis of CNS and to developing neural network physiologically. However, it is reported that NO production and phagocytosis by hyper-activated microglia cause neuronal death in neurodegenerative diseases [3].

Tissue-type transglutaminase (TG2) is ubiquitously expressed in various cells and shows Ca^{2+} concentration-dependent enzyme activity catalyzing to form ε-(γ-glutamyl)lysine isopeptide bond crosslinking between glutamine and lysine residues of proteins [9,10]. TG2 is involved in cell adhesion and construct of cytoskeleton [11]. In addition, extracellular TG2 protein has binding domains to integrin and fibronectin in a Ca^{2+}-independent manner [9,10,12]. These functions contribute to extracellular matrix formation, tissue structures stabilization and epithelia barrier. Moreover, TG2 is reported to play also as a G-protein, protein disulfide isomerase and protein kinase; participating in various intracellular signaling [9,10,13,14]. These various functions of TG2 contribute to proliferation, apoptosis, migration, inflammation, phagocytosis, and so on [9,10,15]. In CNS, several studies show that TG2 is over-expressed in the brains of AD, Parkinson's disease (PD) and Huntington's disease (HD) [9,10]; indicating that TG2 might play important roles in the CNS diseases.

When phagocytes engulf dead/apoptotic cells, they need to interact with "eat me" signal, such as phosphatidylserine (PS), which is exposed on surface of target cells [16,17]. It was reported in peritoneal macrophage that TG2 can bind integrin β_3 and milk fat globule EGF factor 8 protein (MFG-E8), and that TG2 protein is involved in recognition of apoptotic cells; suggesting that TG2 mediates the binding between vitronectin receptor (VR) and MFG-E8 in TG enzyme activity-independent manner [15,18]. TG2 might be associated with microglial phagocytosis as well as peritoneal macrophage. In the present review, we described especially in microglia that TG2 might be involved in the mechanisms of endocytosis, and that the endocytosis might be associated with neurodegenerative diseases.

Transglutaminases

Transglutaminase (TG), a crosslinking enzyme consisting eight isozymes identified, catalyzes Ca^{2+}-dependently to form ε–(γ-glutamyl)lysine isopeptide bond crosslinking between glutamine and lysine residues in proteins or incorporating primary amines at selected peptide-bound glutamine residues [9]. Each TG in mammals is generally localized in a specific tissue and a type of cells, and plays important roles physiologically including skin cornification, blood clotting and wound healing [9]. Among them, TG2 is ubiquitously expressed in various tissues and has various functions as described above contributing to proliferation, apoptosis, migration, inflammation, phagocytosis, and so on [9,10,15]. TG2 is activated in pathological conditions including neurodegeneration, autoimmune diseases, and inflammatory diseases [9,19]. Blood coagulation factor XIII-A (FXIII-A), another TG, is reported to be expressed mainly in microglia in AD patient's postmortem brain [20] and the mutation of

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FXIII might be associated with AD [21]. In monocyte/macrophage, TG2 and FXIII-A are reported to be involved in phagocytosis of apoptotic cells [15,18,22].

Phagocytosis

Endocytosis is generally divided into two categories, pinocytosis and phagocytosis, with size of particle up-taken; pinocytosis is defined as uptake of particles smaller than 1–2 μm and phagocytosis is as uptake over 2 μm [23-25]. In case of scavenging apoptotic cells, phagocytotic cells need to contact with "eat me" signal of target apoptotic cells by their receptors [16,17]. Phosphatidylserine (PS) is known to be a major "eat me" signal, most of which normally exist in inner leaflet of cell membrane; however, when cells are undergone apoptosis, PS is exposed on surface of outer leaflet. Recently, it was reported that PS exposure on cell surface was occurred not only in apoptotic cells but also in activated cells by several stimulation such as intracellular Ca^{2+} elevation [26]. It might cause phagocytosis of viable cells.

An adaptor protein, MFG-E8 consists of four domains, E1 and E2 homologous regions to epidermal growth factor (EGF) and C1 and C2 homologous regions to coagulation factor 8, and proline/threonine rich domain is additionally inserted between them resulting to be called a long-form of MFG-E8 [27]. C2 region of MFG-E8 binds to PS and RGD motif in E2 region of MFG-E8 binds to a VR of macrophage formed by integrin $\alpha_V\beta_{3/5}$ dimer; thereby, macrophage can recognize and uptake the apoptotic cells [28,29]. In peripheral tissues, MFG-E8 protein is mainly released from macrophages and used for phagocytosis [28].

It is reported that microglia also release and utilize MFG-E8 to recognize neurons when stimulated by lipopolysaccharide (LPS) or amyloid β (Aβ) [3,30,31]. It was reported that microglia derived from MFG-E8 knockout mice could endocytose neurons using MFG-E8 containing in the conditioned medium of astrocytes derived from wild-type mice [31]. Therefore, microglia might use MFG-E8 protein derived from both of microglia and astrocytes. In co-culture of neurons and microglia, it is reported that LPS-stimulated NO production from microglia causes exposure of PS on neurons and that microglia recognize neuronal PS as "eat me" signal, resulting to uptake the neurons [30]. The report suggests that excessive NO production and/or facilitation of phagocytosis are thought to be involved in neuronal death.

Involvement of TGs in phagocytosis

It has been reported in astrocytes and microglia that TG2 might participate in inflammatory responses including inducible NO synthase (iNOS) expression and NO production [19,32,33]. Exposure to LPS has been believed to stimulate intracellular signaling pathway through nuclear factor-kappa B (NF-κB) activation [34,35]. In addition, NF-κB binding site is identified on the TG2 promoter region [36]. It was reported that TG enzyme activity is increased by LPS exposure and that activated NF-κB might be involved in TG2 expression [19]. Moreover, Lee et al. reported that inhibitors of TG enzyme activity reduced LPS-induced NO production and that TG2 polymerized IκB (inhibitor of NF-κB) to activate NF-κB; indicating that TG2 augments the NF-κB activation pathway in a self-enhancing manner [19,32].

On the other hand, it has been reported in peritoneal macrophage that TG2 protein is involved in recognition of apoptotic cells [15,18]. It was reported that the phagocytosing activity was decreased in peritoneal macrophage derived from TG2 knockout mice and that TG2 protein interacted with adaptor proteins of PS independently of TG enzyme activity [15]. TG2 was reported to bind each of MFG-E8 and VR which are necessary proteins in the mechanism of phagocytosis [37].

Microglial endocytosis is known to be up-regulated by LPS-stimulation [38] and LPS-simulation in mouse microglial cell line BV-2 increased TG2 expression [39]. In the previous study, we reported in activated microglia that TG2 might be involved in endocytosis of fluorescent beads and dead cells, because of inhibition of the endocytosis by cystamine, an inhibitor of TG enzyme activity [39,40]. Cystamine also inhibited LPS-increased TG2 expression, iNOS expression, and NO production [39]. Therefore, TG2 expression and TG enzyme activity might be closely associated with both NO production and endocytosis in activated microglia.

Phagocytosis in neurodegenerative diseases

It has been reported the association between TGs and neurodegeneration [41-43]. On the other hand, microglial phagocytosis has been reported to be involved in neuronal death in neurodegenerative diseases [44,45]. As described above, in co-culture of neurons and microglia, it is reported that NO derived from LPS-stimulated microglia causes exposure of PS on neurons and that microglia recognize neuronal PS to uptake the neurons [30]. In AD brain, it is known to occur Aβ accumulation and aggregation resulting to senile plaques. It was reported that uptake of soluble Aβ monomer would be observed in resting microglia and the soluble Aβ uptake would not activate microglia [46]; on the other hand, uptake of aggregated Aβ was reported to activate microglia [47]. In neuronal/glial mixed culture, nanomolar Aβ, which was not directly toxic to neurons but activated microglia, induced neuronal death by microglial phagocytosis [3]. It has been reported also in PD model that microglial phagocytosis might be involved in neuronal loss [48-50]. Taken together, the control of microglial phagocytosis might be important for neuronal survival in neurodegenerative diseases and the regulations of TG2 expression and TG enzyme activity might be targets of the mechanism to control microglial phagocytosis.

Conclusion

Engulfment of neurons by activated microglia may play roles in the pathogenesis of neurodegenerative diseases such as AD and PD. TG2 is a cross-linking enzyme, which is activated in AD, PD and HD. In monocyte/macrophage, TG2 is reported to be involved in phagocytosis of apoptotic cells. Also in microglia, TG2 might be involved in endocytosis of invading microorganisms, aggregated proteins, cell debris and damaged cells. Regulations of TG2 expression and TG enzyme activity in microglia might play critical roles in neuronal survival in various CNS diseases through the regulation of endocytosis activity as well as inflammation.

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